Evaluating the Effects of Antagonistic Interactions on Pathogen Inhibition by *Streptomyces* Isolates from a Disease Suppressive Soil

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Dedication

I would like to dedicate this thesis to my parents. Thank you for your love and support. Without you, none of this would have been possible.

Abstract

Diseases of plants threaten global food security and increase the cost of producing food and fiber. Synthetic pesticides have proven effective at suppressing many plant diseases but can require repeated applications at significant expense and cause harm to humans and the environment. Disease suppressive soils often support naturally-occurring soil microbial communities that inhibit plant pathogens and could be used to develop biocontrol or other plant disease management methods. However, high frequencies of resource competition and antagonistic interactions among naturally-occurring pathogen-suppressive populations represent a challenge for reproducing effective microbial disease suppression in agricultural settings. This work sought to further understanding of how the complex network of interactions that occur within disease suppressive soil microbial communities influences pathogen suppression by evaluating pairwise interactions of community members *in vitro*. Specifically, I characterized inhibition and nutrient competition among community members and their relationships to pathogen inhibition. Among a random collection of 75 *Streptomyces* isolates from the rhizosphere soil of potato plants grown in a naturally-occurring scab-suppressive soil in Grand Rapids, MN, 34 isolates were able to inhibit pathogenic *Streptomyces scabies* strain S87. I hypothesized that isolates would have decreased pathogen inhibition when grown *in vitro* with an inhibitory partner isolate relative to when grown alone. Similarly, isolates were hypothesized to have decreased pathogen inhibition when grown with highly nutrient competitive partners relative to when grown alone. However, when pathogen-inhibiting isolates were grown in pairs there were no consistent effects of partner inhibition or

nutrient competition on pathogen inhibition. These results suggest that antagonistic and resource competitive interactions, while potentially important to the long-term establishment of disease suppressive soil microbiomes, may have limited effects on direct inhibition of pathogens. Moreover, this work suggests that successful biological control of plant diseases may not be limited solely to non-antagonistic inoculant mixtures.

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Chapter 1

A Literature Review Regarding the Microbial Ecology and the Functional Roles of Antibiotics in Plant Disease Suppressive Soils

Introduction

Plant diseases can reduce agricultural productivity, thereby threatening food security and the livelihoods of growers and the broader agricultural industry. Conventional agriculture relies on pesticides, biological control, genetic resistance, and cultural practices like rotation and sanitation for reducing losses due to disease. For soil-borne diseases in particular, a potential alternative to costly and sometimes harmful chemical amendments is naturally-occurring disease suppression, as evidenced in disease suppressive soils. Suppressive soils are defined as "soils in which the pathogen does not establish or persist, establishes but causes little damage, or establishes and causes disease for a while but thereafter the disease is less important even though the pathogen may persist in the soil" (Baker & Cook 1974; Weller 1988; Weller *et al.,* 2007). Through the establishment of specific microbial taxa or communities, suppressive soils have been observed to reduce or completely eliminate losses due to disease in multiple plant-pathogen systems. Examples of plant-disease suppressive soils have been documented against many diseases, including: Common scab of potato (*Streptomyces scabies* (Thaxter) Lambert and Loria 1989) (Expósito *et al.,* 2017; Kinkel *et al.,* 2012), Take-all of wheat (*Gaeumannomyces graminis* (Sacc.) Arx & D. L. Olivier) (Kwak & Weller

2013; Shipton 1975), Black root rot of tobacco (*Thielaviopsis basicola* (Berk. & Broome) Ferraris) (Almario *et al.,* 2014; Stutz *et al.,* 1986), Fusarium wilt (*Fusarium oxysporum* Schlechtend.:Fr.) (Weller, Gardener, & Thomashow 2002), Verticillium wilt (*Verticillium dahliae* Kleb.) (Sturz & Christie 2003), Rhizoctonia root rot (*Rhizoctonia solani* J. G. Kühn) (Baker, 1991), White mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) (Rodríguez *et al.,* 2015), Bacterial wilt (*Ralstonia solanacearum* (Smith) Yabuuchi et al.) (Nishiyama *et al.,* 1999), Phytophthora root rot (*Phytophthora capsici* Leonian) (Li *et al.,* 2019), Late blight (*Phytophthora infestans* (Mont.) de Bary) (Orquera-Tornakian *et al.,* 2018), *Heterodera* spp. cyst nematodes (Borneman & Ole Becker 2007; Westphal 2005), *Meloidogyne* spp. root-knot nematodes and *Criconemella* spp. ring nematodes (Pereira da Silva, Medeiros, & Campos, 2018).

There have been suggested to be two distinct types of suppressive soils: general and specific. General disease suppression is often described as a result of inhibitory chemicals produced by the community (like ethylene or hydrogen cyanide), resource competition of community members with the pathogen, and/or changes in environmental conditions that are unfavorable to the pathogen or the development of disease (e.g. changing the soil pH to prevent pathogen spore germination) (Baker & Cook 1974; Hornby 1983; Rovira & Wildermuth 1981). Because the suppressive effect is perceived to be due to the collective activity of the community, attempts to transplant general suppressive soils have been largely unsuccessful (Cook & Rovira 1976; Rovira & Wildermuth 1981). In contrast, specific disease suppressive soils tend to be the result of one or a select few microbial taxa acting against the pathogen. These types of suppressive

soils are reportedly more easily transplanted by directly transferring soil from a suppressive field to a conducive field or by isolating, growing, and inoculating the specific taxa responsible (Kwak & Weller 2013). The distinction between general and specific disease suppressive soils is likely a gross oversimplification of what is probably a spectrum rather than binary pathogen-suppressive characteristics.

Ecology of plant-associated *Streptomyces*

Potato scab suppressive soils serve as excellent model systems for studying how disease suppressive microbial communities work and how they may be induced. In this system, the causal agent of common scab of potato, *Streptomyces scabies*, is suppressed by the production of antibiotics by other members of the genus *Streptomyces*. Rather than a single taxon producing a single antibiotic to inhibit the pathogen (as seen in specific disease suppressive soils), potato scab suppressive soils consist of many taxa capable of inhibiting the pathogen with various antibiotics. The microbial communities of potato scab suppressive soils are hypothesized to be locked in an evolutionary arms race where resource competition has led to the proliferation of a diverse array of antibiotics and resistance phenotypes among successful competitors (Kinkel *et al*., 2012). Furthermore, there is evidence to suggest that niche overlap, genetic relatedness, coevolutionary history, and physical proximity collectively determine the selective pressures that lead to niche differentiation or the evolution of novel inhibitory phenotypes (Kinkel *et al*., 2014; Vaz-Jauri & Kinkel, 2014).

The concept of a niche in ecology often considers the nutrients and physical space (or food niche and place niche) used by an organism (Futuyma & Moreno 1988;

Mikkelson 2005; Pocheville 2015). Niche overlap is a measure of the specific resources or other environmental variables used by two organisms. In microbial ecology, the number of nutrients that can be utilized by two microbial isolates can be used as a measurement of niche overlap (Kinkel *et al*., 2014; Vaz-Jauri & Kinkel, 2014). In terms of long-term adaptation and evolution, it is hypothesized that pairs of organisms experiencing greater niche overlap will also experience stronger selection pressure for either niche exclusion or niche differentiation (Carlson & Taffs, 2010). In the context of soil *Streptomyces*, niche exclusion might consist of inhibiting a competitor and niche differentiation might consist of one of the competitors adapting to utilize a nutrient that the other does not (Kinkel *et al*., 2011). It has also been hypothesized that in environments with a lower overall diversity of nutrients, taxa have less opportunity to niche differentiate and therefore there is higher selection pressure to niche exclude (Kinkel *et al*., 2011). Within agriculture, these hypotheses have been extended to suggest that long-term monocultures, producing a lower diversity of nutrients than more diverse plant communities, will result in stronger selection pressure for niche exclusion, for example, by selection for novel inhibitory phenotypes. There is evidence to support this idea from observations of greater inhibition intensity among sympatric *Streptomyces* isolates with higher niche overlap than among sympatric isolates with lower niche overlap (Essarioui *et al*., 2017; Kinkel *et al*., 2014).

Phylogenetic relatedness is a measure of approximate similarity between the genomes of two taxa. While it could be hypothesized that more recently-diverged taxa are more similar and are therefore more likely to have higher niche overlap, several studies

have actually found no significant relationship between genetic relatedness (based on 16S sequence, box-PCR, and/or fatty acid profiling) and phenotypic traits like nutrient use or inhibition among *Streptomyces* spp. (Davelos *et al.,* 2004) and among *Pseudomonas* spp. (Lottmann & Berg, 2001). However, it is likely that the methods used to measure genetic relatedness in those studies were not sufficiently sensitive. Use of 16S sequence or comparably coarse genotyping methods may be incapable of differentiating taxa until they have diverged so far as to have significantly different inhibition and nutrient use phenotypes. Higher resolution analyses, like multi-locus sequence analysis or whole-genome sequencing, could reveal correlations between genetic relatedness and phenotypic traits like nutrient use and inhibition.

The physical distance between two microbes ultimately determines the degree to which they interact and influence one another. Generally, the greater the spatial distance between populations, the less influence they have on each other in terms of access to nutrients, physical space for growing, antibiosis, or signaling. Over time, species interactions affect long-term adaptations among coexisting populations. Two populations in close physical proximity, influencing each other for extended periods of time, are more likely to experience adaptations in response to interactions with each other. For example, if one population evolves a novel antibiotic that inhibits a nutrient competitor, the competing population might evolve a new form of the antibiotic's target and evade the novel antibiotic. This back-and-forth of reciprocal genetic change describes their coevolutionary history. But in order for there to be a history of coevolution between two

taxa, they have to be physically close enough to each other to interact and influence one another's fitness.

In general, since a large portion of the nutrients available to soil microbial communities are originally derived from their plant hosts/neighbors, it is unsurprising that aspects of the plant community (e.g. species richness, diversity, and productivity) have enormous influence on soil microbes like *Streptomyces*. Plants have been observed to selectively enrich certain microbes through the secretion of specific root exudates (Badri *et al.,* 2009). Plants can also indirectly affect the nutrients flowing to the microbial community by influencing neighboring plants positively (e.g. induced plant defenses) or negatively (i.e. allelopathy) (Bais *et al*., 2006). Soil nutrient amendments used in agriculture, including nitrogen, phosphorus, and potassium, can directly influence the microbial community or indirectly influence the microbes by altering plant growth (Bünemann *et al*., 2006).

Signaling within microbial communities can induce changes in metabolic processes like nutrient use and production of secondary metabolites (Egland *et al*., 2004; Keller & Surette, 2006). In the same way that populations can exert selection pressure for novel inhibitory phenotypes, it has been hypothesized that there is selection for signals that can mediate microbial interactions like nutrient competition and inhibition. Vaz-Jauri & Kinkel (2014) showed significant support for this hypothesis by observing increased rates of altered inhibition by *Streptomyces* when grown in the presence of a sympatric vs. allopatric *Streptomyces* isolate, suggesting species-specific signaling to mediate production of antibiotics among competitors. *Streptomyces* isolates were also more likely

to exhibit altered inhibition when grown in the presence of a partner with high niche overlap, with high genetic relatedness, or that were inhibited by the isolate (Vaz-Jauri & Kinkel 2014). These data showed that signaling and induction of changes in inhibitory phenotypes was more likely among sympatric, co-evolved, highly related *Streptomyces*. Earlier work by Davelos *et al.,* (2004) testing the inhibition and resistance of 153 *Streptomyces* isolates against a test collection of 10 isolates showed significant spatial variation in inhibitory phenotypes, suggesting that the fitness benefits of specific inhibitory phenotypes are not consistent across locations. However, there was no significant relationship between resistance phenotype and location, suggesting that maintenance of resistance has lower fitness costs than that of inhibition and that the selection pressures for resistance and inhibitory phenotypes may differ (Davelos *et al.,* 2004). Together, these observations suggest a competitive community of soil *Streptomyces* evolving to inhibit or evade their neighbors and kin in response to environmental conditions like nutrients and space. For the purposes of inducing and/or maintaining suppressive soils, this could mean encouraging these microbes to adapt and maintain novel inhibitory phenotypes by controlling nutrient diversity and abundance and by facilitating the ongoing ecological and evolutionary interactions between highly competitive microbes.

Dynamic roles of antibiotics in disease suppressive soils

Antibiotic-mediated inhibition of pathogens is the center of much disease suppressive soils research (Bonanomi *et al.,* 2010; Keel *et al.,* 1992; Voisard *et al.,* 1989; Weller *et al.,* 2007). However, antibiotics do more than just inhibit pathogens; antibiotics

can also influence community dynamics by imposing spatial structure or by acting as signals (Romero *et al*., 2011). In suppressive soils, the roles of antibiotics can be divided into microbe-pathogen and microbe-microbe (where "microbe" refers to a non-pathogenic member of the soil microbial community).

Microbe-pathogen antibiotic interactions

One of the most well-studied models of a suppressive soil microbial community is in Take-all of wheat. In this system, after a few years of continuous wheat monoculture, the incidence of take-all, caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici*, increases until it can devastate entire fields of wheat (Kwak & Weller 2013). Then, after several years of continuous monoculture, the incidence of take-all suddenly declines, until the disease becomes almost non-existent under disease-favorable conditions and non-existent under disease-unfavorable conditions for as long as the monoculture is maintained. The suppression of *G. graminis* has been connected to fluorescent *Pseudomonas* spp. and the production of the antibiotics 2,4'-diacetylphloroglucinol (DAPG) and pyrrolnitrin for quite some time (Cook & Rovira 1976; Weller *et al.*, 2007). In this specific disease suppressive soil, the presence of DAPG-producing soil bacteria is the key to suppression. Transplanting take-all suppressive bacteria to successfully recreate a suppressive soil has been demonstrated several times (Cook & Rovira 1976; Raaijmakers & Weller 1998; Shipton 1975).

Another example of the suppressive microbial community directly inhibiting the pathogen with antibiotics is black root rot of tobacco, caused by the fungal pathogen

Thielaviopsis basicola. In this system, DAPG-producing *Pseudomonas protegens* CHA0 is believed to inhibit the *T. basicola*, despite sufficient levels of colonization (10^4 CFU/g root) for disease suppression being observed on plants in conducive soils (Stutz *et al.,* 1986). DAPG was found to inhibit growth of *T. basicola in vitro* and in soil tests and DAPG-producing Pseudomonads were found in black root rot suppressive soils at densities comparable to those found in take-all suppressive soils (Keel *et al.,* 1990, Keel *et al.,* 1992; Stutz *et al.,* 1986). Two other chemicals with antibiotic properties, pyoluteorin and hydrogen cyanide, are also released by DAPG-producers in black root rot suppressive soil communities, but have been found to be insufficient for pathogen suppression in the absence of DAPG (Keel *et al.,* 1992; Ramette *et al.,* 2006; Voisard *et al.,* 1989).

Microbe-microbe antibiotic interactions

Microorganisms can communicate in many different ways. First, there are countless chemicals produced by microbes with specific receptors and overall function. These signal chemicals can be intended for conspecific communication, like acyl-homoserine lactones for quorum-sensing, or they can be sensed by other microbes, like cis-11-methyl-2-dodecenoic acid produced by *Stenotrophomonas maltofilia* that induces biofilm formation by *Pseudomonas aeruginosa* (Romero *et al.,* 2011; Ryan & Dow 2008). In both cases, the chemicals trigger specific altered behavior in the receiving microbes.

The second way that microbes can communicate is through detection of molecular waste or byproducts from a neighbor microbe. In this case, the signaling chemicals aren't expressly produced for the purpose of signaling. Rather, these chemicals are produced and left behind as part of other processes (e.g. growth, motility, metabolism) and then those microbes within a certain proximity of the signal producer, detect the chemicals and alter their behavior.

A third method of microbial communication is with antibiotics. Antibiotics are unique from quorum-sensing or other strictly defined signal chemicals, because there are three possible outcomes from exposure to an antibiotic that can be dependent upon concentration: recipient grows unaffected, recipient dies, or recipient alters behavior. For the first outcome, if the recipient lacks the target of the antibiotic, has a sufficient resistance mechanism (e.g. an efflux pump), or the concentration of the antibiotic is too low, then the recipient can survive exposure to the antibiotic. For the second outcome, if the recipient has the target, lacks resistance, and the concentration is high enough, then the recipient dies. Finally, for the third outcome, if the recipient experiences some combination of a non-lethal target receptor, a sufficient resistance mechanism, and/or the concentration is too low, then the recipient can survive and alter its behavior, potentially to improve its fitness in response to the detected signal (e.g. by shifting its preferred nutrient use to avoid competition with the signal producer). The concentration of the antibiotic at the responding microbe is mediated by the production rate, the population size, and/or the distance from the signaling microbe.

With respect to *Streptomyces*, subinhibitory concentrations of antibiotics have been shown to impact growth, nutrient use, and niche width (Vaz-Jauri *et al.*, 2013). The type and magnitude of responses to specific antibiotics varied among isolates, but tetracycline, vancomycin, and rifampicin (all originally derived from soil bacteria) induced significant increases in growth with the highest frequency (Vaz-Jauri *et al*., 2013). Importantly, these findings suggest a specific role of antibiotics at subinhibitory concentrations in mediating nutrient competition among soil bacteria like *Streptomyces*. It is also interesting to note that antibiotics at subinhibitory concentrations could influence nutrient competition short-term, serving as a form of regulation on the selection pressure that nutrient competition exerts on the evolution of novel antibiotics (Schlatter & Kinkel, 2014).

Conclusions

Suppressive soils hold significant promise as both sources of disease suppressive microbes and models for studying microbial ecology. In order to better understand disease suppressive soils, further systematic study of the effects of antagonistic interactions, nutrient competition, and spatial proximity on inhibition of a plant pathogen by both individual antagonists and antagonist combinations is required. Conventional wisdom might suggest combining biocontrol inoculants that do not compete for resources or antagonize each other because if the inoculants are starving and/or killing each other, it could negatively impact overall pathogen suppression. However, the potential for sympatric inhibition and nutrient competition occur commonly among populations from disease suppressive soils and are theorized to be central to the evolution and maintenance

of pathogen inhibition (Kinkel *et al*., 2014; Schlatter *et al*., 2017). To optimize mixed microbial inoculants for biocontrol, further study is required to determine how hypothesized drivers of long-term evolution, including antagonism and nutrient competition, affect pathogen inhibition among antagonist combinations.

Chapter 2

Evaluating the Effects of Antagonistic Interactions on Pathogen Inhibition by *Streptomyces* Isolates from a Disease suppressive Soil

Introduction

Diseases of plants caused by fungal, bacterial, and viral pathogens result in reduced agricultural productivity, which can threaten growers' livelihoods and have downstream impacts on commercial supply chains and food security. Conventional agriculture commonly relies on crop rotation, sanitation, resistance breeding, synthetic pesticides, and other management practices to prevent losses due to plant diseases. A potential alternative to chemical control of pathogens is the development of disease suppressive soils.

Disease suppressive soils support naturally-occurring microbial communities that suppress crop disease, usually by inhibiting pathogens (Baker & Cook, 1974; Raaijmakers & Weller, 1998; Weller *et al*., 2007). In the case of potato common scab, there are several species of *Bacillus*, *Rhizoctonia*, and *Streptomyces* that have been observed to suppress the pathogen and reduce or prevent scab lesions from forming on otherwise susceptible potatoes (Larkin, 2020; Lin *et al*., 2018; Lorang *et al*., 1989; Sarwar *et al*., 2019; Zhang *et al*., 2020). The disease suppressive capacity of these microbial communities is hypothesized to result from a "coevolutionary arms race", in which nutrient competition results in strong selective pressures for inhibitory phenotypes against saprophytic competitors (Kinkel *et al*., 2012). The inhibition of the potato scab

pathogen is considered a consequence of inhibitory dynamics within the saprophytic soil community (Kinkel *et al*., 2012; Kinkel *et al*., 2014).

Many attempts to recreate the disease suppression observed in naturally-occurring disease suppressive soils have been made, most often by isolating and inoculating pathogen-inhibiting microbes (Liu *et al*., 1995; Schlatter *et al*., 2017). However, inoculation of one or a few antagonistic microbes may substantially underestimate the complexity of naturally-occurring disease suppressive communities. Moreover, there is little known regarding the effects of combining microbial inoculants to mimic the complexity of disease suppressive soil communities and the consequences for disease suppression. Specific questions include: Does combining microbial inoculants capable of inhibiting each other result in decreased pathogen suppression relative to the individual inoculants? Does combining microbial inoculants with high niche overlap result in decreased growth, decreased antibiotic production, and/or decreased pathogen inhibition? That is, while the high frequencies of inhibition and niche overlap within disease suppressive soil microbial communities are hypothesized to be critical to the development of the suppressive capacity, the immediate effects of these antagonistic interactions on pathogen suppression is not known.

This study sought to determine the effects of interactions among antagonistic populations from a naturally-occurring disease suppressive soil on the *in vitro* inhibition of a target pathogen. The specific objectives were to: 1) determine the types and frequencies of interactions (e.g. inhibition and nutrient competition) and genetic relatedness among populations in a naturally-occurring disease suppressive soil

community, and 2) evaluate how resource competition and antagonistic interactions between isolates influence pathogen inhibition by each isolate. We hypothesized that: 1) isolates will exhibit reduced capacities to inhibit a pathogen in the presence of an inhibitor, and 2) isolates will exhibit reduced capacities to inhibit a pathogen in the presence of a strong competitor for nutrients.

Methods

Streptomyces isolates

Streptomyces spp. isolates were collected from the rhizosphere soil of potato plants grown in a potato scab suppressive soil at the University of Minnesota North Central Research and Outreach Station in Grand Rapids, MN, USA (Kinkel, unpublished). This suppressive soil was continuously planted to potato as part of a common scab resistance breeding program run by potato breeders at the University of Minnesota, beginning in 1943 (Lorang *et al*., 1989). By 1965, potato scab disease severity had begun to decline, and in 1971 the plot was abandoned for potato scab breeding purposes due to a complete lack of symptoms on susceptible hosts (Lorang *et al*., 1989). In 1997, rhizosphere soil from potato plants grown in this suppressive soil was sampled, serial dilutions of each sample were plated onto oatmeal agar (OA) medium (Kuster, 1959), and *Streptomyces* spp. were visually identified for isolation (Kinkel, unpublished). Pathogenic *Streptomyces scabies* strain S87 was originally isolated from scab lesions on a potato tuber grown in Minnesota (Liu, 1992). Spore suspensions were kept frozen in 20% glycerol at -20°C and -80°C for short- (≤ 6 months) and long-term (> 6 months) storage, respectively.

Seventy-five isolates (25 from each of three locations within a disease suppressive field in Grand Rapids, MN) were screened in triplicate for their ability to inhibit the pathogen isolate *S. scabies* S87 as described in Vidaver *et al*., (1972). Briefly, 10 µL of spore suspensions of candidate pathogen inhibitors at $10^6 – 10^8$ CFU/mL were pipetted as a dot onto Petri plates containing 20 mL of starch-casein-agar (SCA) medium and incubated at 28°C for 72 hours. Then, the plates were each inverted over a watch glass containing chloroform in a chemical fume hood for 1 hour to kill the bacteria. Plates were set in biosafety cabinets for 30 minutes to allow the chloroform to dissipate and then a second layer of 10 mL of SCA was pipetted on top of the original layer. After the second layer of SCA solidified, the pathogenic isolate *S. scabies* S87 was spread on the plate and incubated at 28°C for 72 hours to form a lawn. The presence of an inhibition zone (area of *S. scabies* S87 lawn unable to grow in the proximity of the now dead candidate pathogen-inhibitor colony) indicated that a given candidate isolate was inhibitory. Finally, among isolates capable of inhibiting *S. scabies* S87, 10 were randomly chosen from each of the three locations within the disease suppressive field, providing the 30 isolates used for all subsequent analyses. Isolates originally collected from the same location were considered sympatric.

Nutrient use characterization among Streptomyces isolates

To determine the nutrients that an isolate was capable of utilizing for growth, nutrient use profiles for every isolate were developed using Biolog® SF-P2 plates (Biolog, Inc., Hayward, CA, USA), as described in Davelos *et al.,* (2004). Briefly, cultures were grown on Petri plates containing OA medium for 7 days, after which spores

and vegetative growth were collected by sterile swab and transferred into a 0.2% carrageenan solution. Suspensions were adjusted to OD_{590} = 0.22 and then 1.5 mL of the suspension was added to 13.5 mL of 0.2% carrageenan to make a final volume of 15 mL with a suspension of approximately 1 x 10^7 CFU/mL. Biolog® plates were loaded with 100 µL of this suspension into each well. Loaded plates were incubated at 28°C and growth was measured once per day on days 3 – 7 by absorbance at 590 nm (abs_{590}) using a Biotek® Synergy™ microplate reader (Winooski, VT, USA). Nutrients on which an isolate was able to grow to an $abs_{590} > 0.005$ after blank subtraction (here, the abs_{590} of a no-growth control well was used as the blank).

Niche width was defined as the number of nutrients that an isolate could utilize for growth. Niche overlap was defined as the sum of the quotients of growth by two isolates on the tested nutrients (where the maximum quotient for any given nutrient was 1), divided by the niche width of the isolate being overlapped. The formula for how much isolate A overlapped with isolate B was defined as: Overall niche overlap $_{\text{A over B}} = \frac{\sum_i^n \frac{\min \text{abs}_{\text{A,B}}}{\text{abs}_{\text{B}}}}{n}$, where there are "$n$" nutrients upon which isolate B was able to grow ($abs_B > 0.005$; aka "niche width"), "abs_{B}" is the abs_{590} of isolate B when grown on the i^{th} nutrient, and "$\min \text{abs}_{\text{A,B}}$" is the lesser of a pair of isolates' absorbance values (abs_A and abs_B) on the i^{th} nutrient. For example, if on the first nutrient tested ($i = 1$) isolate A grew to abs_{590} = 0.2 and isolate B grew to abs_{590} = 0.4, then isolate A would overlap isolate B by 50% on that nutrient (because $\frac{0.2}{0.4}$ = 50%) and isolate B would overlap isolate A by 100% on that nutrient (because $\frac{0.2}{0.2}$ = 100%). The overall niche overlap for isolate A

against isolate B would then be determined by adding all the quotients and then dividing by the niche width of isolate B (n). The asymmetry of the niche overlaps represents a theoretical competitive advantage derived from an individual being capable of utilizing more nutrients and/or each nutrient more efficiently on average.

To simplify comparisons among isolates, niche overlap values were divided into discrete categories of "low", "medium", and "high" nutrient competition. These categories were determined by dividing the total range of observed niche overlap values among all 30 isolates into three equal ranges (approx. 0.23 – 0.47, 0.48 – 0.72, and 0.73 – 0.97). If isolate A had an overall niche overlap against isolate B of 0.5, then isolate A was deemed to have a "medium" niche overlap against isolate B.

Finally, mean growth efficiency (MGE) was calculated for each isolate by averaging an isolate's growth (abs_{590} after blank subtraction) across all the nutrients that the isolate could utilize for growth ($abs_{590} > 0.005$).

Non-metric multidimensional scaling (NMDS) of the isolates based on their nutrient utilization was calculated using the "vegan" package (Oksanen *et al*., 2019) and visualized using the "ggplot2" package (Wickham, 2016) in R (R Core Team, 2019). The similarity of isolates from the same location was compared to the similarity of isolates between locations by applying an analysis of similarity (ANOSIM) test to the dissimilarity matrix created from the isolates' growth measurements (matrix created using Bray-Curtis dissimilarity). The R-value calculated in the ANOSIM test can range from -1 to +1, where an R-value = 0 means the groups were indistinguishable and an R-

value = 1 means that the groups were completely distinct. This analysis tests the null hypothesis that the similarity among isolates from different locations is equal to, or greater than, the similarity among isolates from the same location.

Analysis of pairwise inhibition among sympatric Streptomyces

To determine inhibitory interactions among sympatric isolate pairs (45 unique pairs for each group of 10 isolates), a modified version of the double-layer agar method described by Vidaver *et al.,* (1972) was used. Briefly, the stock spore suspensions were made for each isolate by collecting $10^6 – 10^8$ CFU/mL from solid oatmeal agar media cultures using sterile cotton-tipped applicators. Petri plates containing 20 mL of starch-casein-agar (SCA) media were dotted with 10 µL of three different isolates' spore suspensions ($10^6 – 10^8$ CFU/mL) and then incubated at 28°C for 72 hours. Next, individual plates were inverted over a watch glass containing 10 mL of chloroform and maintained in a chemical fume hood for 1 hour to kill dotted isolates. Plates were then set in biosafety cabinets for 30 minutes to allow chloroform to dissipate, and a second layer (10 mL) of SCA was pipetted on top of the medium. After the second layer of SCA solidified, 50 µL of spore suspension of the target isolate was spread on the plate and incubated at 28°C for 72 hours to form a "lawn". The presence of an area where the lawn of target isolate was unable to grow was used to determine that the target isolate was inhibited. For any two isolates, there were three possible types of inhibitory relationship: mutually inhibitory, one-way inhibitory, or mutually non-inhibitory. Overall, all three inhibitory relationship types were observed, however not all isolates had partners of all three inhibitory types (i.e. some isolates only had mutually non-inhibitory relationships

with all nine of their sympatric partners, so we were unable to evaluate those isolates' responses to being grown with inhibitory partners). All isolates were qualitatively screened for inhibition against all sympatric isolates twice.

Effects of Co-plating on Pathogen Inhibition

To determine the effects of the presence of another isolate on the capacity of each strain to inhibit a pathogen (*S. scabies* S87), two colonies were plated by dotting 10 µL each of 10^6 – 10^8 CFU/mL of suspension onto SCA plates at 1, 3, or 5 cm apart. Plates were then incubated at 28°C for 72 hours. Every isolate was grown in pair with every sympatric isolate or with a clonal colony at all three distances, each replicated three times. Sympatric partner isolates were categorized based on inhibitory relationship and niche overlap category. Thus, there were six partner types: partners that do or do not inhibit and have "low", "medium", or "high" niche overlap against the focal isolate. Every isolate was also grown alone in the center of a plate replicated three times as a control for determining "colony area" and "inhibition area" against *S. scabies* S87 when grown without a partner. After incubation, the colonies were killed with chloroform, overlaid with SCA, and a lawn of *S. scabies* S87 was established as previously described. After incubating at 28°C for 72 hours, plates were imaged using a Canon EOS Rebel T6 camera mounted to a mechanical arm with constant height from the imaging surface. A 10 cm ruler was included in every image to standardize measurements. Colony areas and zone of inhibition areas for the colonies on each plate were measured using the circle tool in the FIJI image analysis program (Schindelin *et al.*, 2012).

16S rRNA DNA Sequencing and Analysis

The 16S rRNA sequences of the 30 pathogen inhibitors were determined as previously described (Schlatter *et al*., 2013). Briefly, pure cultures were grown in liquid YEME media (Kieser *et al*., 2000), pelleted, washed with TE buffer, and then DNA was extracted using the DNeasy® UltraClean® Microbial Kit (Qiagen®, Hilden, Germany). Following DNA extraction, the 16S rRNA was amplified using the 27F primer (AGAGTTTGATCCTGGCTCAG) and 1391R primer (GACGGGCRGTGWGTRCA). Amplified DNA was then sent to the University of Minnesota Genomics Center for Sanger sequencing. Forward and reverse sequences for each isolate were aligned using the alignment tool in the Benchling software program (Benchling.com) and then taxonomy was assigned to the consensus (aligned) sequence using BLAST blastn suite for nucleotides (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were read using the "seqinr" package (Charif & Lobry, 2007) into R (R Core Team, 2019) and genetic distances were calculated using the ClustalW algorithm in the "msa" package (Bodenhofer *et al.*, 2015) as part of Bioconductor 3.10. These analyses were used to evaluate hypothesized relationships between genetic relatedness, nutrient use, and inhibition among microbial community members. Phylogenetic trees were constructed from the genetic distance matrices using the neighbor-joining method (Saitou & Nei, 1987) and rendered using "ggtree" (Yu *et al.*, 2017), a dependency of "ggplot2" (Wickham, 2016).

Results

Inhibitory profile characterization

Among the 25 isolates from each location, the numbers of pathogen-inhibiting isolates ranged from 10 – 14 (Table 1). Among the 10 pathogen-inhibiting isolates chosen at random from each of the three locations, the mean pathogen inhibition area differed significantly among locations (Fig. 1a, $F(2,27) = 7.91$, $p = 0.002$, ANOVA). Specifically, isolates from location GS02 had significantly greater pathogen inhibition than those from location GS10 (Tukey's HSD; $\alpha = 0.05$). Isolates from location GS04 did not differ significantly from isolates from locations GS02 or GS10 pathogen inhibition area.

Despite differences in pathogen inhibition, there were no significant differences in inhibition of sympatric isolates among locations (Fig. 1b, $F(2,27) = 0.26$, $p = 0.773$, ANOVA). However, the number of sympatric isolates inhibited varied widely among isolates (range 0-9 isolates inhibited).

There are three possible types of inhibitory relationship to describe isolate pairs: mutually non-inhibitory (neither isolate inhibits the other); one-way inhibitory (only one of the isolates inhibits the other); or mutually inhibitory (both isolates inhibit each other). Overall, mutually-inhibitory pairs were the least common, represented by 13 pairs out of the total 135 pairs (9%). Mutually non-inhibitory and one-way inhibitory pairs were approximately equally well-represented with 59 (44%) and 63 pairs (47%), respectively (Table 1).

Nutrient use characterization

Across all locations, the distributions of isolate's niche widths, niche overlaps and mean growth efficiencies were similar (Figs. 1c-e). Overall, isolates had niche widths ranging from 56 to 94 nutrients (Table 1). Mean niche widths were not significantly

different among locations (Fig. 1c, $F(2,27) = 0.998$, $p = 0.382$, ANOVA). The mean niche overlap that isolates experienced from sympatric partners ranged from 0.376 – 0.886, indicating that some isolates experienced very low niche overlap from their sympatric partners on average (0.376), while others experienced very high niche overlap from their sympatric partners on average (0.886). Mean sympatric niche overlap did not vary significantly among locations (Fig. 1d, $F(2,27) = 0.319$, $p = 0.73$, ANOVA).

The overall range of niche overlap values observed among all isolates' against their partners ranged from 0.23 – 0.97 (Table 1). Discrete categories of "low", "medium", or "high" nutrient competition (corresponding with isolates that had partners with niche overlap values of 0.23 – 0.47, 0.48 – 0.72, or 0.73 – 0.97 against them) show that isolates experiencing "low" nutrient competition from a partner were the least common, represented by only 45 out of 270 pairs (17%), while isolates experiencing "medium" and "high" levels of nutrient competition from their partners were approximately equally well-represented with 114 (42%) and 111 (41%) pairs, respectively (Table 1).

Mean growth efficiency (MGE), or the average growth of an isolate on all the nutrients it was able to utilize, ranged from 0.068 to 0.307 (Supp. Table 1). Locations GS02, GS04, and GS10 had mean MGE values of 0.166, 0.138, and 0.160, respectively, and were not significantly different among locations (Fig. 1e, $F(2,27) = 0.766$, $p = 0.474$, ANOVA).

Finally, nutrient use profiles were compared using NMDS (Fig. 2). When compared statistically using ANOSIM (where $R = 0$ indicates the groups are

indistinguishable and R = 1 indicates the groups are completely distinct), isolates' nutrient use profiles did not differ significantly among locations (Fig.2, R = 0.09289, p = 0.03, ANOSIM).

Genetic characterization

Among isolates, 16S sequences clustered into 2 large clades, each containing multiple isolates from each location (Fig. 3). All 30 isolates were identified as belonging to 10 *Streptomyces* species using NCBI BLAST with at least 99.5% sequence identity (Supplemental Table 5).

To test the hypothesis that more closely-related isolates have more similar inhibitory phenotypes, the correlation between the difference in the number of sympatric isolates inhibited and the genetic dissimilarity of isolates was measured. No correlation between genetic dissimilarity and the number of sympatric isolates that an isolate inhibits was found (Fig. 4a, $\rho = 0.208$, $p = 0.130$, Spearman's Rank-Order Correlation). Similarly, no correlation between genetic dissimilarity and pathogen inhibition intensity was found (Fig. 4b, $\rho = 0.243$, $p = 0.649$, Spearman's Rank-Order Correlation). Thus, genetic relatedness was not a predictor of inhibitory phenotypes like the number of sympatric isolates that an isolate could inhibit or pathogen inhibition intensity.

To test the hypothesis that more closely-related or co-evolved isolates are more likely to induce changes in each other's pathogen inhibition, the relationship between the genetic dissimilarity of a partner isolate and the change in pathogen inhibition when grown with that partner was evaluated. A small, but statistically significant, amount of the variation in percent change in inhibition due to being grown with a partner was

explained by genetic dissimilarity from that partner (Fig. 4c, $R^2 = 0.0036$, $p = 0.00131$). The isolates had greater increases in pathogen inhibition when grown with isolates that were less genetically similar (when measured by 16S sequencing).

Finally, we evaluated whether more closely-related isolates have more similar nutrient use profiles by characterizing the relationship between genetic dissimilarity and niche overlap for all pairs of isolates. No significant correlation between genetic dissimilarity and niche overlap was found (Fig. 4d, $\rho = -0.023$, $p = 0.789$, Spearman's Rank-Order Correlation). Furthermore, nutrient use by isolates belonging to the two major clades observed in the phylogenetic tree did not differ significantly (Fig. 5, $R = -0.021$, $p = 0.623$, ANOSIM).

Evaluation of the effects of microbial interactions on inhibition of a plant pathogen in vitro

To test the effects of a partner isolate on the ability of each isolate to inhibit a pathogen, the 30 pathogen-inhibiting isolates were grown with sympatric isolates in all possible pairwise combinations. Overall, neither nutrient competition, nor being grown with an inhibitory partner, had a consistently beneficial or detrimental effect on an isolate's ability to inhibit a pathogen. Moreover, the presence of any type of partner (based on inhibitory relationship and degree of niche overlap) rarely had any effect on an isolate's ability to inhibit a pathogen. When grown with non-inhibitory partners, mean pathogen inhibition did not change significantly from that observed when isolates were grown alone (Fig. 6a, 95% Confidence Interval = -0.071 – 0.241, one-sample two-sided Student's T-test). Likewise, when grown with inhibitory partners, mean pathogen

inhibition did not change significantly as compared with inhibition when grown alone (Fig. 6b, 95% CI = -0.103 – 0.140, one-sample two-sided Student's T-test). Overall, when isolates were grown with a sympatric partner, pathogen inhibition was equally likely to increase or decrease, regardless of whether the partner was inhibitory or not (p = 1 for increase vs. decrease when grown with inhibitory partners, p = 0.108 for increase vs. decrease when grown with non-inhibitory partners, Binomial Tests).

Similarly, there were no consistent effects of strong resource competition on pathogen inhibition. The mean change in pathogen inhibition area for isolates grown with a weakly competitive partner did not differ significantly from 0 (Fig. 7a, "low" niche overlap 95% CI = -0.280 – 0.207, one-sample two-sided Student's T-test). Likewise, the mean change in pathogen inhibition area when grown with a moderately or highly competitive partner did not differ significantly from 0 (Fig. 7b, "medium" niche overlap 95% CI = -0.094 – 0.160; Fig 7c "high" niche overlap 95% CI = -0.038 – 0.132; one-sample two-sided Student's T-tests). When compared to being grown alone, isolates were equally likely to exhibit an increase or a decrease in pathogen inhibition area when grown with a sympatric partner of any level of nutrient competition (p = 0.754 for low nutrient overlap partners, p = 0.728 for medium nutrient overlap partners, p = 0.627 for high nutrient overlap partners, Binomial Tests).

Next, the effects of both partner inhibition and nutrient competition on an isolate's pathogen inhibition were evaluated. Based on the two levels of inhibitory relationship ("inhibited", "not inhibited") and three levels of niche overlap ("low", "medium", "high"), there were six partner types. The frequencies of the six possible

partner types observed by combining sympatric isolates from a disease suppressive soil were: 12% not inhibited – "low", 31% not inhibited – "medium", 24% not inhibited – "high", 4% inhibited – "low", 11% inhibited – "medium", and 18% inhibited – "high" partners. Mean change in pathogen inhibition when grown with any of the six partner types did not differ significantly from 0 (Fig. 8, all 95% CI's include 0, one-sample two-sided Student's T-test). When compared to being grown alone, isolates were equally likely to exhibit an increase or decrease in pathogen inhibition area when paired with any type of partner ($p \geq 0.180$ for all six partner types, Binomial Tests).

Finally, the effects of distance between partners on an isolate's pathogen inhibition were evaluated. With one exception, isolates were equally likely to exhibit an increase or decrease in pathogen inhibition area when paired with any type of partner at any of the tested distances ($p \geq 0.039$, Binomial Tests, $\alpha = 0.00278$ to correct for family-wise error). Only partners that did not inhibit, had "high" niche overlap, and were grown 1 cm apart (the shortest distance tested) were more likely to result in an increase in pathogen inhibition area ($p = 0.002$, Binomial Test, $\alpha = 0.00278$ to correct for family-wise error). No type of partner, at any distance, was more or less likely to induce a decrease in pathogen inhibition area by the focal isolate.

Further analyses considered the characteristics of the isolates that induced a change in the pathogen inhibition of their partner isolates. Among the 30 isolates, 13 failed to induce an increase in pathogen inhibition area of 50% or greater in any of their nine partners; 12 isolates induced an increase in pathogen inhibition area of 50% or greater in one of their nine partners; and five isolates induced an increase in pathogen

inhibition area of 50% or greater in at least two of their nine partners (Fig. 9a). Thus, while some isolates stimulate an increase in pathogen inhibition in multiple partners, most (25 of 30) isolates had little effect on pathogen inhibition by other isolates. Isolates that induced large increases in pathogen inhibition did not exhibit significant differences in the number of sympatric isolates inhibited, pathogen inhibition intensity, niche width, or growth efficiency, from isolates that did not induce changes in pathogen inhibition (Supp. Table 1).

In pairs where large changes in pathogen inhibition were observed, the characteristics of the responsive isolates were compared. A distribution of the isolates based on the number of partners that induced a large increase in their pathogen inhibition showed that most isolates (19 of 30) never responded to their partners with an increase in pathogen inhibition of 50% or greater, while six of 30 isolates exhibited this increase with one partner, and five of 30 isolates exhibited this increase with two or more partners (Fig. 9b). Of the six isolates that exhibited a large increase (>50%) in pathogen inhibition in response to a partner, five had below average pathogen inhibition when grown alone, and four exhibited a large increase with multiple partners (Supp. Table 2). Notably, one isolate (GS04-02) exhibited an increase in pathogen inhibition of 50% or greater when grown with six of its nine partners. Similarly, all three isolates that had a large decrease (>50%) in pathogen inhibition in the presence of a partner had below average pathogen inhibition when grown alone, and all three exhibited large decreases with multiple partners (Supp. Table 3).

Discussion

To combat plant diseases, modern agriculture relies on plant breeding for host resistance, cultural practices, pesticides along with biological control. The microbial communities of disease suppressive soils provide a potential source of significant, broad-spectrum antagonism of many soilborne pathogens and often populated by mutually-inhibitory pathogen antagonists (Köberl *et al*., 2013; Nagórska *et al*., 2007). This study sought to further understanding of how antagonistic and competitive interactions and genetic similarity influence pathogen inhibition by isolates from a disease suppressive soil. By evaluating isolates from a disease suppressive soil in pairwise combinations, this study was able to characterize the types and frequencies of interactions among antagonistic populations and to evaluate the influences of these interactions on the isolates' ability to inhibit a pathogen.

There was little spatial differentiation in pathogen or sympatric inhibition, nutrient use, or 16S sequence among *Streptomyces* isolates from distinct locations within the disease suppressive soil field. Further, isolates did not cluster by location based on 16S rRNA; rather the isolates clustered into two large clades, each of which was well-represented in each sampling location (Fig. 3). While the 16S rRNA sequence has been argued to have insufficient resolution to reliably differentiate species within the *Streptomyces* genus, a relationship between sequence similarity and phenotypic traits, including nutrient use or inhibitory profile, has been observed in some studies (Guo *et al*., 2008; Vaz-Jauri & Kinkel, 2014). However, no relationship was observed between genetic dissimilarity and inhibitory traits or nutrient use in this work (Figs. 4a-c). In theory, more similar 16S rRNA sequences could represent a more recently diverged

lineage and therefore more similar isolates. However, in this study, the 16S rRNA sequence alone was insufficient to predict the similarity of such complex phenotypes.

Effects of microbial interactions on pathogen inhibition

Previous work (Kinkel *et al*., 2012) showed tremendous diversity in antagonistic populations in the potato scab-suppressive soil studied here. To effectively capitalize on knowledge of suppressive soils for agriculture, it is necessary to have a thorough understanding of how individual antagonists interact with each other (Compant *et al*., 2019; Trabelsi & Mhamdi, 2013), and the implications for pathogen suppression. In this study, pathogen inhibition was measured for sympatric isolates grown in 135 unique pairwise combinations, enabling thorough evaluation of the effects of different inhibitory and nutrient use interactions on pathogen suppression *in vitro*. There was little evidence that the presence of an inhibitory and/or highly nutrient competitive partner resulted in consistent decreases in pathogen inhibition at any of the tested distances (Fig. 6, Fig. 7). This suggests that combining potential inoculants that inhibit each other does not necessarily result in reduced disease suppression. While there were a few isolates that induced large changes in multiple partners, their relationships to those partners (e.g. inhibitory vs. non-inhibitory, highly vs. minimally competitive, closely vs. distantly related) were varied and inconsistent (Supp. Table 1). In total, these data suggest that inhibition or nutrient competition between antagonists may have little direct effect on pathogen suppression. However, the potential for resource competition or inhibitory interactions between antagonists to influence soil colonization or growth dynamics, with implications for pathogen suppression *in vivo*, remains unknown.

Within this study, just over half of the isolates were capable of inducing a change in the pathogen inhibition of another isolate (Fig. 9a). The observation that most isolates were able to induce a shift in at least one partner isolate may suggest that most isolates produce signals that can enhance pathogen suppression by partners. In this study, 10 isolates (one-third) exhibited changes in pathogen inhibition in response to at least one partner, with one isolate responding to six of its nine partners (Fig. 9b, Supp. Table 4), suggesting variation in responsiveness of isolates to partners, consistent with previous research (Vaz-Jauri & Kinkel, 2014). Similar observations have been made regarding microbial signaling in suppression of *Sclerotium rolfsii* by a combined microbial inoculant consisting of *Pseudomonas* strain PHU094, *Trichoderma* strain THU0816, and *Rhizobium* strain RL091 (Singh *et al*., 2013b). While many of the environmental conditions required for microbial community-mediated disease suppression are well understood (e.g. suitable temperature, moisture, and soil pH), relatively little is known regarding the complexity of signaling as it relates to pathogen suppression (Duffy *et al*., 1996; Sarma *et al*., 2015; Singh *et al*., 2013a; Stockwell *et al*., 2011).

Isolates from a naturally-managed prairie soil have been observed to be more likely to alter inhibitory phenotypes in response to a sympatric partner than an allopatric partner (Vaz-Jauri & Kinkel, 2014). Specifically, *Streptomyces* isolates were more likely to show decreased inhibition when paired with distantly related isolates than when paired with more closely related isolates. Unlike the study of Vaz-Jauri & Kinkel (2014), this study did not test allopatric combinations. However, among the sympatric combinations tested in our study, there was no correlation between genetic similarity and change in

pathogen inhibition. The lack of correlation between genetic similarity and altered inhibition observed in this study may have differed from the results previously observed by Vaz-Jauri & Kinkel (2014) because our study was based on isolates collected from an intensively managed monoculture where the microbial community is mixed via tillage, while the isolates used in Vaz-Jauri & Kinkel (2014) were originally collected from a non-tilled prairie soil. The effects of soil mixing due to tillage in the potato field may have cancelled out any effects spatial distance on the microbial communities, effectively making them all allopatric to each other despite being collected in the same soil sample.

Many of the isolates that exhibited a large change in pathogen inhibition had below average pathogen inhibition when grown alone (Supp. Table 2, Supp. Table 3). This may suggest that isolates with weak antibiosis against the pathogen (when grown alone) may be more likely to be influenced by a partner isolate when growing in proximity to another isolate. However, there was no significant correlation between pathogen inhibition when grown alone and the change in pathogen inhibition when grown with a partner (Supp. Fig. 1b). Furthermore, fewer than half of the isolates with below average pathogen inhibition exhibited a change in pathogen inhibition of 50% or greater. Pathogen inhibition area when grown alone was insufficient to predict how isolates will respond to being grown with partners.

Conclusions

This study found that there was no consistent effect of niche overlap or partner inhibition on the ability of an isolate to inhibit a pathogen. Importantly, the results of this study suggest that inhibitory or competitive combinations should not necessarily be

avoided when making mixed microbial inoculants. A better understanding of the strain-specific interactions taking place within the soil microbial community, including inhibition, nutrient competition, and interspecies signaling, is necessary to fully take advantage of disease suppressive soils.

Illustrations
Tables

Table 1 Nutrient use and inhibition characteristics of *Streptomyces* isolates collected from three different locations (GS02, GS04, and GS10) within a disease suppressive soil in Grand Rapids, MN, USA. First, the number of isolates capable of inhibiting the plant-pathogenic *Streptomyces scabies* strain S87 out of 25 isolates from each location. Then, among the 10 randomly selected pathogen-inhibitors from each location: the number of each inhibition pair type observed between sympatric isolates for each location (mutually non-inhibitory, one-way inhibitory, or mutually inhibitory); the minimum, median, and maximum niche widths observed for the isolates from each location; the minimum, median, and maximum niche overlaps observed among sympatric isolates for each location; the number of each nutrient competition pair type ("low", "medium", or "high") observed among sympatric isolates for each location.

	Location			
Characteristic	**GS02**	**GS04**	**GS10**	**Sum**
Number of S87-Inhibiting Isolates (out of 25 per location)	14	10	10	34
Number of Mutually Non-Inhibitory Pairs	20	20	19	59
Number of One-Way Inhibitory Pairs	16	21	26	63
Number of Mutually Inhibitory Pairs	9	4	0	13
Minimum Niche Width	66	63	61	
Median Niche Width	85.5	80.5	82	
Maximum Niche Width	94	92	94	
Minimum Niche Overlap	0.28	0.23	0.32	
Median Niche Overlap	0.70	0.66	0.65	
Maximum Niche Overlap	0.97	0.94	0.90	
Number of Low Nutrient Competition Pairs	15	18	12	45
Number of Medium Nutrient Competition Pairs	30	34	50	114
Number of High Nutrient Competition Pairs	45	38	28	111

Figures

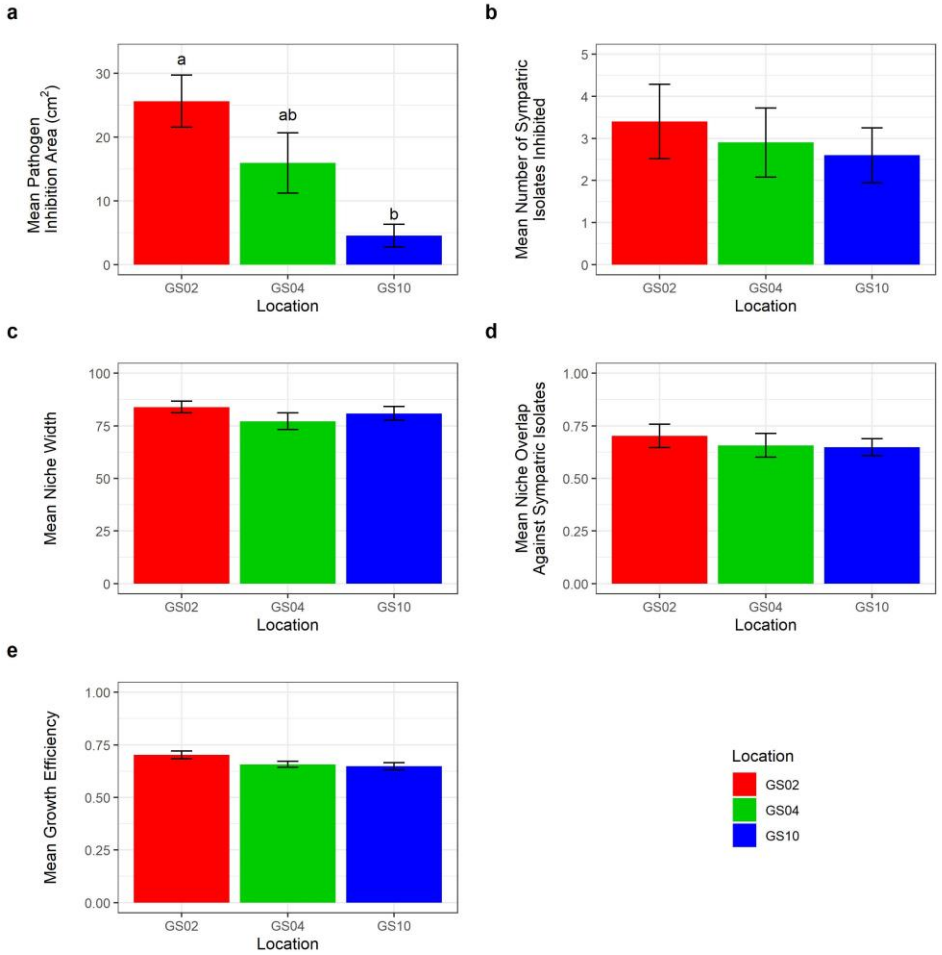


Fig. 1 **a)** Mean pathogen inhibition area (cm^2) for isolates from each location ($F(2,27) = 7.91$, $p = 0.00198$, ANOVA). **b)** Mean number of sympatric isolates inhibited by isolates from each location ($F(2,27) = 0.26$, $p = 0.773$, ANOVA). **c)** Mean niche width for each location ($F(2,27) = 0.998$, $p = 0.382$, ANOVA). **d)** Mean niche overlap against sympatric partners for isolates from each location ($F(2,27) = 0.319$, $p = 0.73$, ANOVA). **e)** Mean growth efficiency for isolates from each location ($F(2,27) = 0.766$, $p = 0.474$, ANOVA).

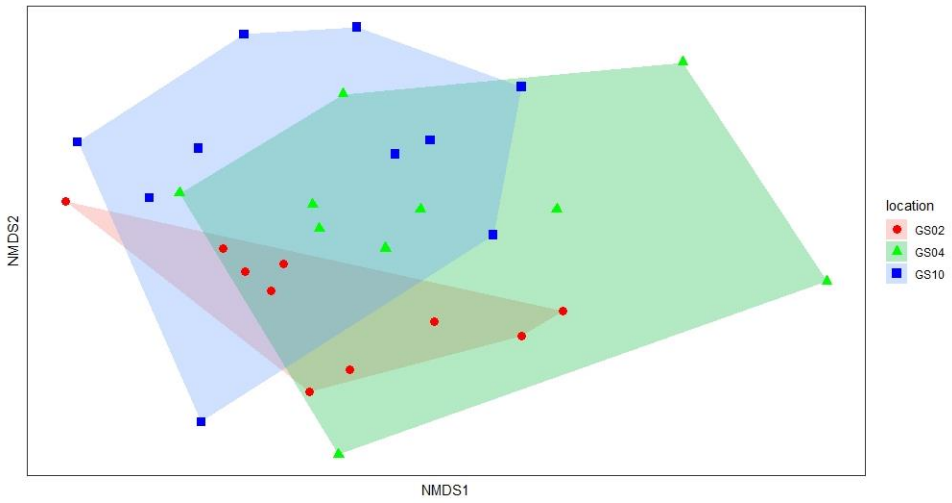


Fig. 2 Non-metric multidimensional scaling (NMDS) of nutrient use by 30 *Streptomyces* isolates obtained from a naturally-occurring disease suppressive soil grouped by location. Each symbol represents an independent isolate obtained from one of three locations (GS02, GS04, GS10). NMDS values were based on each isolate's ability to grow on 95 different nutrients, where points that are closer represent isolates with more similar nutrient utilization. Isolates from the three locations did not vary significantly in terms of nutrient utilization (R = 0.09289, p = 0.03, ANOSIM).

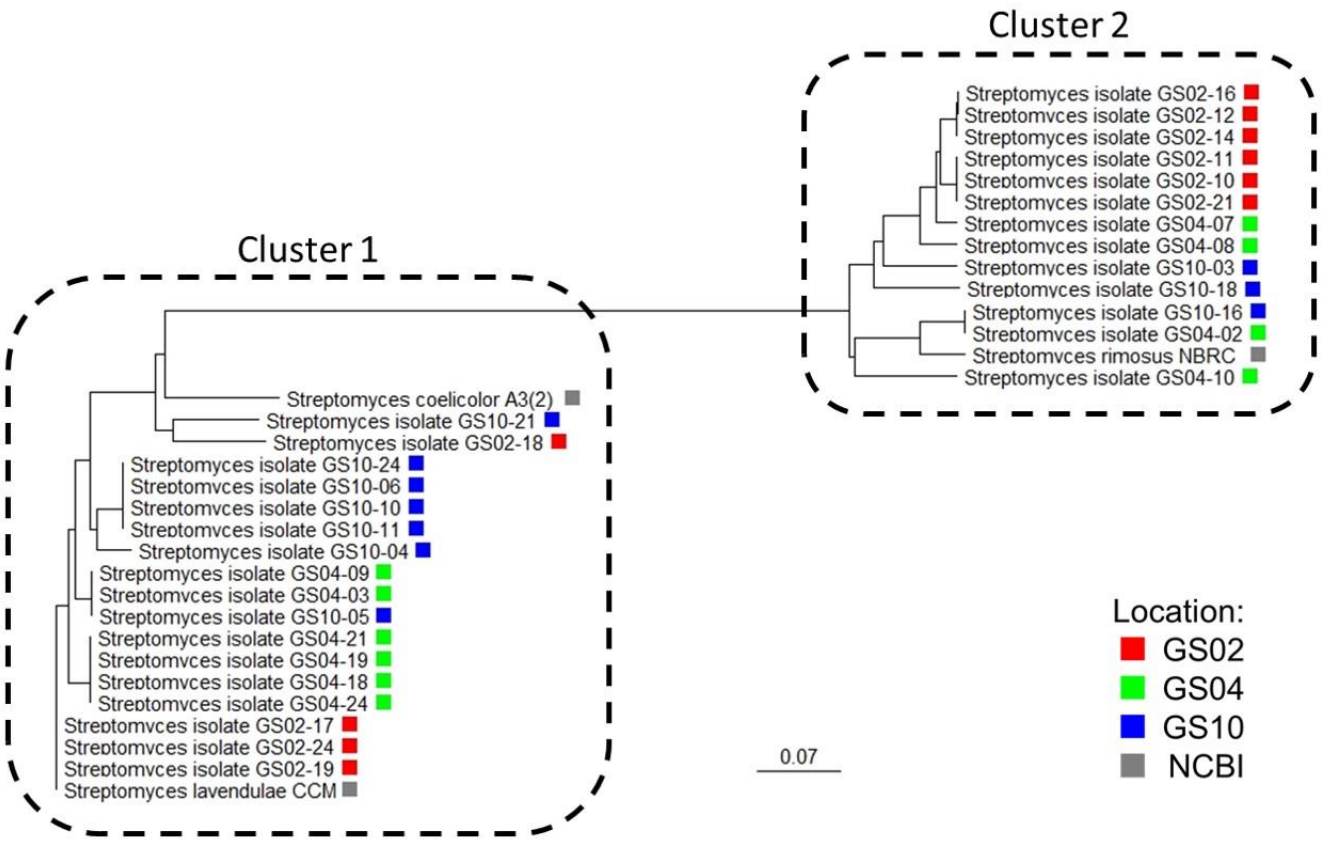


Fig. 3 Phylogenetic tree constructed from 16S rRNA DNA sequences using the neighbor-joining method. Each isolate from the Grand Rapids suppressive soil is color-coded by its location of origin. Publicly-available sequences for *Streptomyces rimosus* strain NBRC, *Streptomyces coelicolor* strain A3(2), and *Streptomyces lavendulae* strain CCM 3239 from the National Center for Biotechnology Information (NCBI) included for reference. Scale bar represents genetic distance in base pair substitution frequency (i.e. 7 bp substitutions for every 100 bp) between isolates was calculated using the ClustalW multiple sequence alignment algorithm.

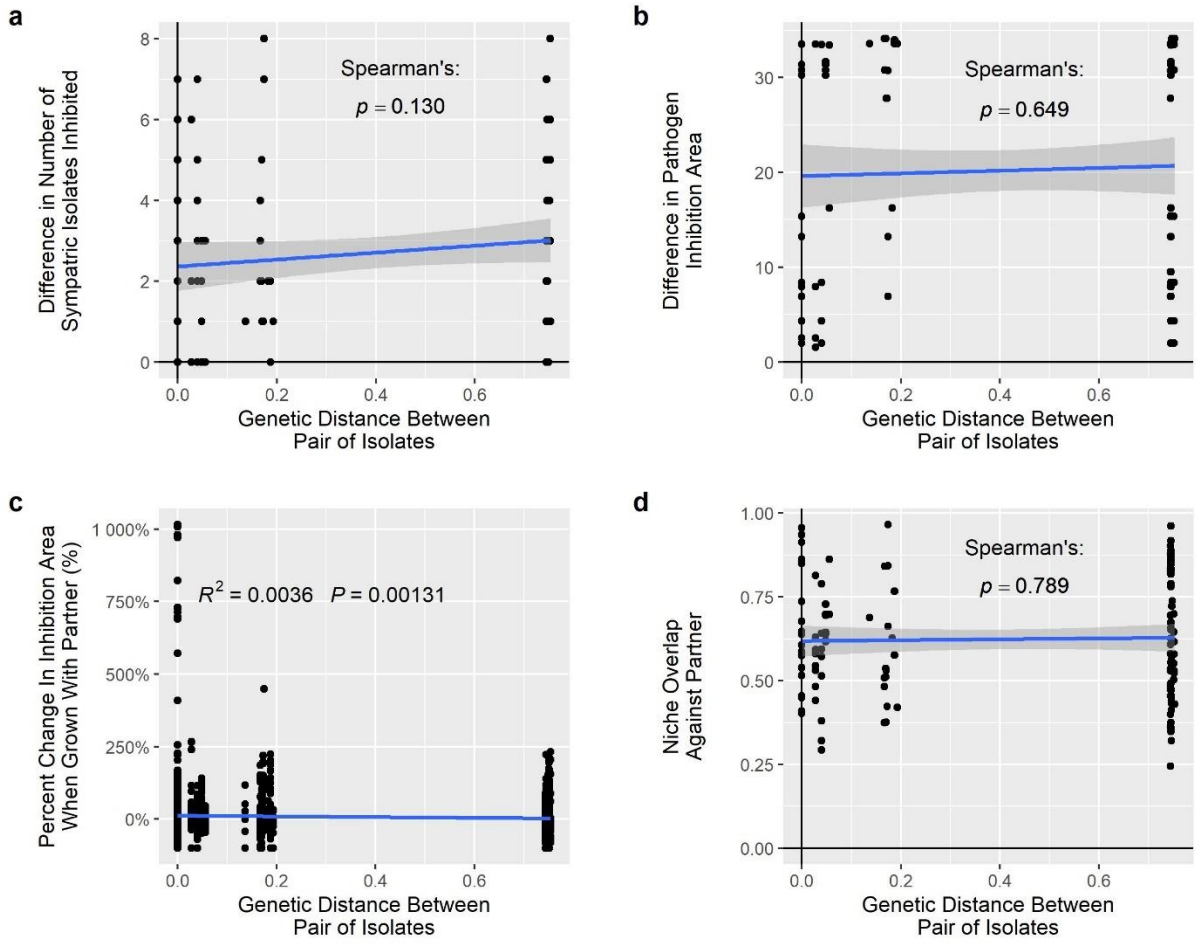


Fig. 4 Relationships for genetic distance (16S dissimilarity as calculated by the ClustalW algorithm) between all possible isolate pairs and **a)** the difference in the number of sympatric isolates they inhibit (ρ = 0.208, R^2 = 0.0036), **b)** the difference in their pathogen inhibition areas when they are grown alone (ρ = 0.243, R^2 = 0.649), **c)** the change in pathogen inhibition area when grown alone vs. when grown with the partner (R^2 = 0.0036, p = 0.001), or **d)** the difference in their niche overlaps (ρ = -0.023, p = 0.789).

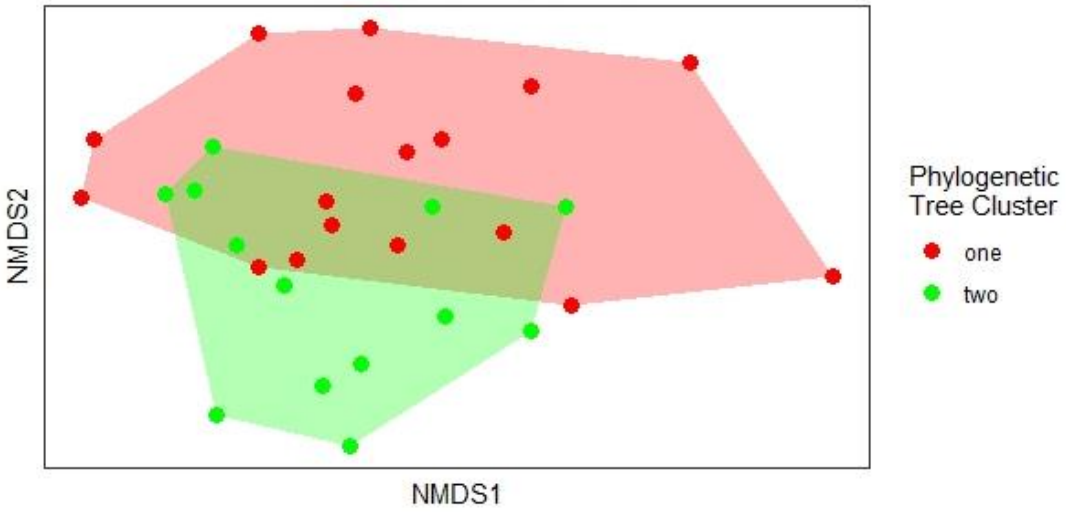


Fig. 5 Non-metric multidimensional scaling (NMDS) analysis of nutrient use by *Streptomyces* isolates from a naturally-occurring disease suppressive soil grouped by major phylogenetic cluster, as shown in figure 4 (n = 17 and 13 for clusters one and two, respectively). Each point represents an isolate's growth on 95 different nutrients in the SF-P2 Biolog® Plate, where points that are closer represent isolates with more similar nutrient utilization. The two clusters of isolates did not significantly vary in terms of nutrient use (R = -0.021, p = 0.623, ANOSIM Test).

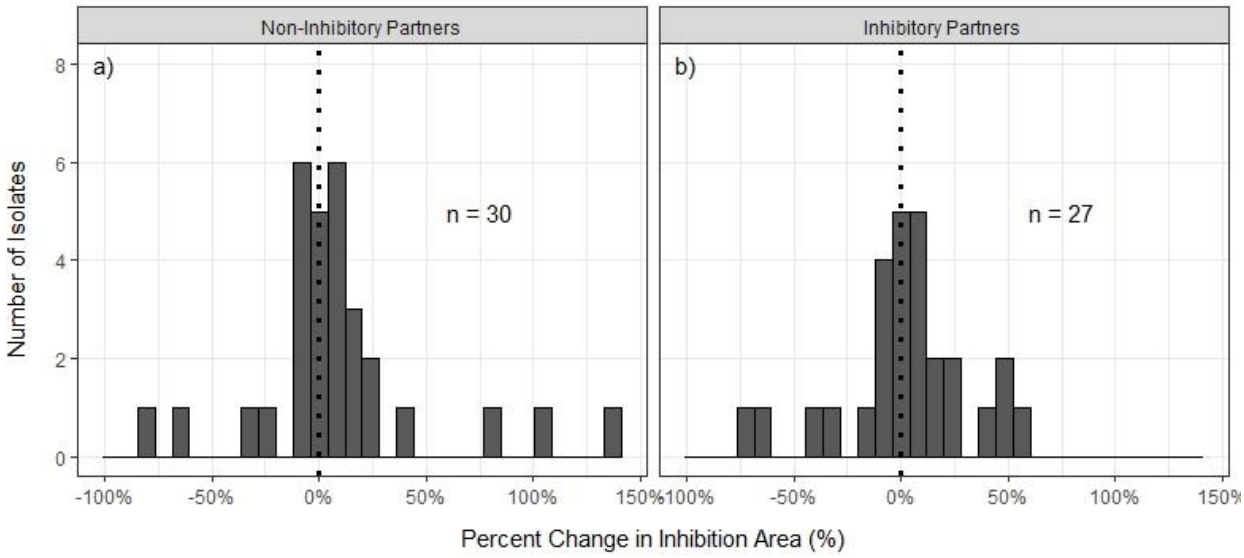


Fig. 6 Distribution of isolates' average change in inhibition area when grown in the presence of non-inhibitory **(a)** vs. inhibitory partners **(b)**. Bars represent the number of isolates that had the respective percent change in pathogen inhibition area. Dotted lines indicate no change (0%) in mean inhibition area when grown alone vs. when grown with inhibitory vs. non-inhibitory partners. All 30 isolates tested had at least one partner that did not inhibit them and 27 of the 30 isolates tested were inhibited by at least one partner isolate.

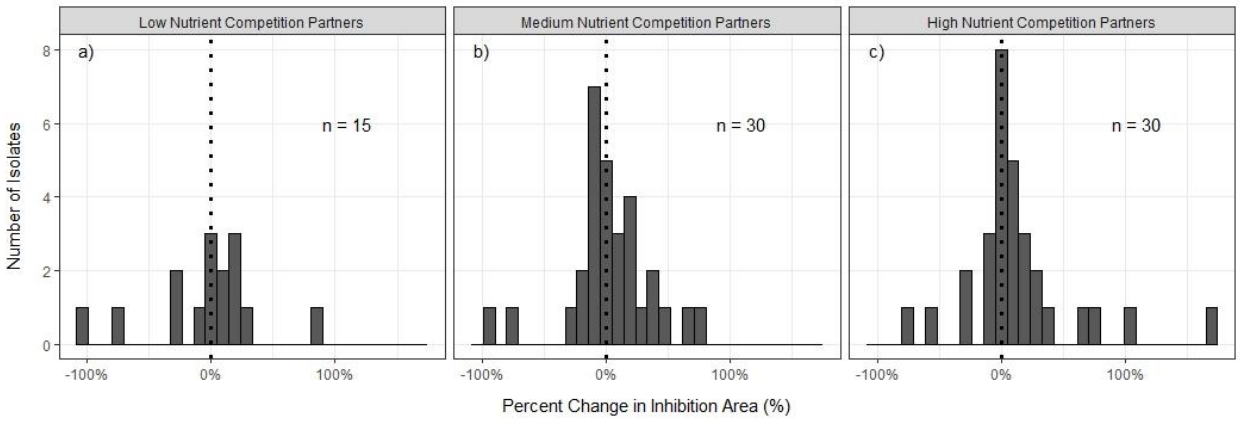


Fig. 7 Distribution of isolates' average change in inhibition when grown in the presence of partners with varying niche overlap intensities ("low", "medium", and "high" nutrient competition). Bars represent the number of isolates that had the respective percent change in pathogen inhibition area. Dotted line indicates no change (0%) in inhibition area when grown alone vs. when grown with partners of varying niche overlap. All 30 isolates tested had at least one partner with "medium" **(7b)** and "high" **(7c)** niche overlap against them, however only 15 of the 30 isolates tested had at least one partner with "low" **(7a)** niche overlap against them.

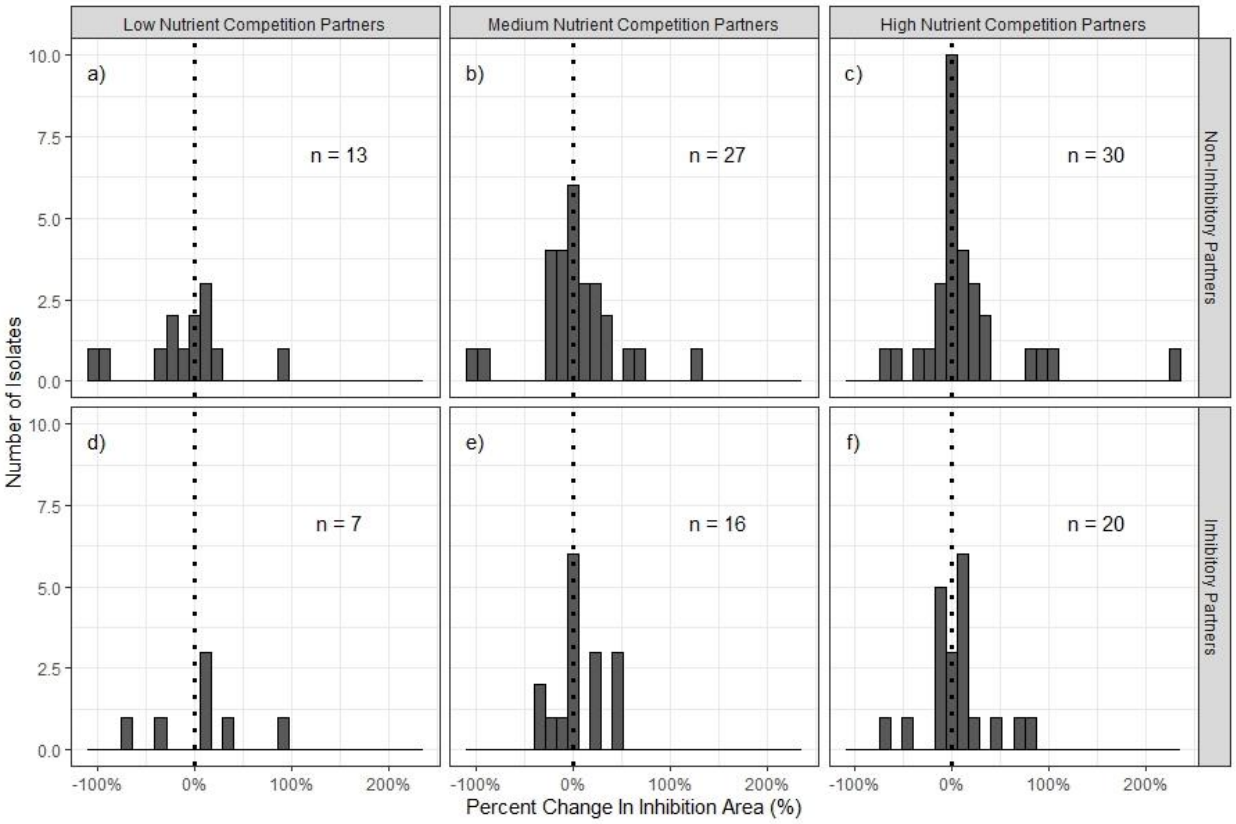


Fig. 8 Distribution of isolates' average change in inhibition when grown with partners that did inhibit **(a-c)** or did not inhibit **(d-f)** them and had varying niche overlap intensity against them. Bars represent the number of isolates that had the respective percent change in pathogen inhibition area when grown with the respective partner types. Dotted lines indicate no change (0%) in mean inhibition area when isolate was grown alone vs. with partner(s) of the varying types.

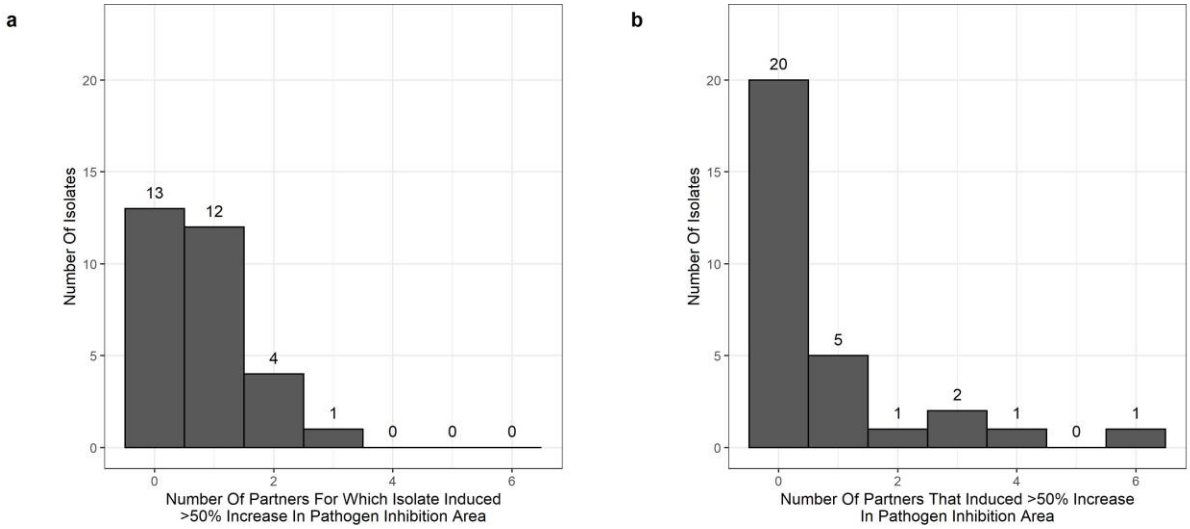


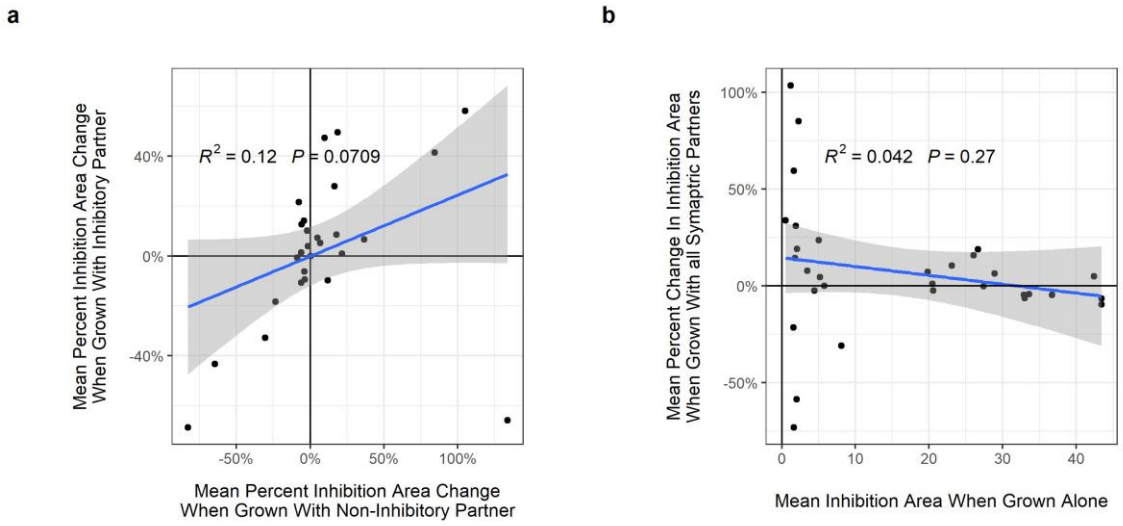
Fig. 9 **a)** Number of isolates that induced an increase in mean pathogen inhibition area greater than 50% in 0-3 of their sympatric partner isolates. **b)** Number of isolates that exhibited increases in their mean inhibition area of greater than 50% in the presence of 0-6 of their 9 sympatric partner isolates.

Supplemental Table 1 Characteristics of 30 *Streptomyces* isolates from a disease suppressive soil sorted by the number of partners for which they induced a large change in pathogen inhibition. Characteristics include the number of partners for which the isolates induced an increase in pathogen inhibition area of greater than 50%, the number of sympatric isolates that the isolates inhibited, the mean pathogen inhibition area when the isolates were grown alone (cm^2), the niche width, and the mean growth efficiency.

Isolate	Number of Partners for which Isolate Induced >50% Increase in Pathogen Inhibition Area	Number of Sympatric Isolates Inhibited	Mean Pathogen Inhibition Area when Grown Alone (cm^2)	Niche Width	Mean Growth Efficiency
GS10-21	3	1	1.39	94	0.23
GS04-10	2	1	7.68	88	0.24
GS10-05	2	3	2.05	87	0.12
GS10-11	2	5	4.12	80	0.11
GS10-18	2	1	1.91	84	0.25
GS02-11	1	2	37.98	79	0.13
GS02-14	1	4	38.01	81	0.14
GS02-19	1	2	28.55	66	0.10
GS04-03	1	1	1.86	63	0.07
GS04-07	1	2	1.89	85	0.12
GS04-18	1	1	1.99	81	0.17
GS04-19	1	6	39.78	80	0.14
GS04-21	1	8	37.47	92	0.16
GS04-24	1	5	27.07	56	0.10
GS10-06	1	3	3.81	70	0.11
GS10-10	1	4	5.21	75	0.14
GS10-24	1	3	4.70	78	0.16
GS02-10	0	2	35.47	77	0.15
GS02-12	0	2	33.90	80	0.10
GS02-16	0	8	1.94	94	0.17
GS02-17	0	2	25.96	90	0.19
GS02-18	0	9	4.76	90	0.31
GS02-21	0	2	27.54	92	0.20
GS02-24	0	1	22.26	90	0.17
GS04-02	0	0	2.29	65	0.14
GS04-08	0	2	19.22	72	0.10
GS04-09	0	3	20.12	90	0.14
GS10-03	0	0	0.49	90	0.18
GS10-04	0	6	20.07	90	0.12
GS10-16	0	0	1.53	61	0.18

Supplemental Table 2 Summary information regarding isolates used from the locations GS02, GS04, and GS10. Taxonomy based on BLAST search of the NCBI GENBANK database using 16S rRNA DNA sequences amplified using primers 27F (AGAGTTTGATCCTGGCTCAG) and 1391R (GACGGGCRGTGWGTRCA). Percent sequence identity was determined by percentage of nucleotides that matched identity and location.

Isolate	Taxon Based on 16S Sequence	Sequence Identity
GS02-10	Streptomyces lavendulae strain NBRC	99.92%
GS02-11	Streptomyces lavendulae strain NBRC	99.92%
GS02-12	Streptomyces colombiensis strain NRRL	99.84%
GS02-14	Streptomyces lavendulae strain NBRC	99.84%
GS02-16	Streptomyces colombiensis strain NRRL	99.92%
GS02-17	Streptomyces lavendulae strain NBRC	99.92%
GS02-18	Streptomyces mirabilis strain NBRC	99.84%
GS02-19	Streptomyces lavendulae strain NBRC	99.92%
GS02-21	Streptomyces lavendulae strain NBRC	99.92%
GS02-24	Streptomyces lavendulae strain NBRC	99.92%
GS04-02	Streptomyces glebosus strain LMG	99.85
GS04-03	Streptomyces spororaveus strain NBRC	99.92%
GS04-07	Streptomyces spororaveus strain NBRC	100%
GS04-08	Streptomyces avidinii strain NBRC	100%
GS04-09	Streptomyces spororaveus strain NBRC	99.92%
GS04-10	Streptomyces rishiriensis strain NRRL	99.76%
GS04-18	Streptomyces colombiensis strain NRRL	99.84%
GS04-19	Streptomyces colombiensis strain NRRL	99.77%
GS04-21	Streptomyces colombiensis strain NRRL	99.77%
GS04-24	Streptomyces colombiensis strain NRRL	99.84%
GS10-03	Streptomyces pratensis strain ch24	99.92%
GS10-04	Streptomyces avidinii strain NBRC	100%
GS10-05	Streptomyces virginiae strain T34	100%
GS10-06	Streptomyces spororaveus strain NBRC	99.69%
GS10-10	Streptomyces spororaveus strain NBRC	99.77%
GS10-11	Streptomyces spororaveus strain NBRC	99.77%
GS10-16	Streptomyces glebosus strain LMG	99.92%
GS10-18	Streptomyces gardneri strain NBRC	99.53%
GS10-21	Streptomyces rishiriensis strain NRRL	99.77%
GS10-24	Streptomyces spororaveus strain NBRC	99.69%



Supplemental Fig. 1 **a)** Correlation between mean percent change in an isolate's pathogen inhibition area when grown with an isolate that does inhibit it vs. when it was grown with an isolate that does not inhibit it. Linear regression fit with $R^2 = 0.12$ and $p = 0.0709$. **b)** Correlation between the mean percent change in an isolate's pathogen inhibition area when paired with all sympatric partners and its mean pathogen inhibition area when grown alone. Linear regression fit with $R^2 = 0.042$ and $p = 0.27$.

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